Composition Studies on Tobacco XXVI.

Aryl Amines In the Nitromethane-Soluble Neutral Fraction of Smoke Condensate

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Introduction

Recent studies on the composition of the nitromethane-soluble, neutral substances in cigarette smoke condensate have resulted in the isolation of several previously unidentified constituents: benzyl benzoate (8); benzyl cinnamate (8); cinnamonitrile (6); and myristicin (5-allyl-2,3-methylenedioxyphenyl methyl ether) (7). In addition, indole, carbazole and derivatives thereof were found in this fraction (8) and evidence was obtained for the presence of cinnamyl cinnamate, styryl cinnamate and an aromatic halogenated hydrocarbon probably derived from an insecticide (6). The present study is a continuation of this work and concerns the isolation of several aryl amines from the nitromethane-soluble fraction: diphenylamine, 9,9-dimethylacridan[1], *N*-phenyl-4-isopropylphenylamine and *N*-phenyl-2-naphthylamine.

Methods

Smoke condensate (1 kg) obtained by mechanical smoking of domestic 85 mm nonfilter cigarettes (50,000) was fractionated as previously described (6). In this method, acids and bases are removed by conventional solvent partitioning procedures. Subsequently, the neutral substances are partitioned among methanol-water (80:20 $^{v}/_{v}$), cyclohexane and nitromethane. The yield of nitromethane solubles is about 3.5-5.0 percent of the original condensate.

For isolation of the aryl amines, the nitromethane-solubles (39 g) were initially separated on activated (9) silicic acid (0.8 kg). The column was eluted with benzene-petroleum ether (1:4), benzene, ether-benzene (1:4), ether and methanol. The ether-benzene eluate yielded the bulk (68 percent) of the eluted material. This eluate was further separated on neutral alumina (Grade I) by successive elution with benzene-petroleum ether (3:10 followed by 1:1) and other solvents. The aryl amines were removed by 1:1 benezene-petroleum ether and the residue (1.6 g) in this eluate was again chromatographed (Column A) on neutral alumina. Column A was eluted with a series of solvents from benzene-petroleum ether (1:4) to methanol.

Each eluate from Column A was evaporated to a residue and screened by the previously described gas chromatographic procedure using an SE-30 column (7). In some cases, separations of components in the eluates were made by thin layer chromatography on silicic acid using petroleum ether-ether-acetic acid (78:20:2) as the developing solvent and a sulfuric acid-sodium dichromate spray as a nonspecific detecting reagent.

Results and Discussion

Gas chromatographic separation of the benzene-petroleum ether (1:4) eluate of Column A revealed more than 50 peaks. Of these, three were collected for further study. The first of these (elution time = 18.5 min.) had an infrared spectrum which was identical with that of authentic diphenylamine (DPA). The mass spectrum of the unknown gave a parent peak at 169 and the fragmentation pattern was similar to DPA. Cochromatography of the isolated compound and authentic DPA on the SE-30 column and on thin layer plates gave a single peak or spot, respectively. The unknown compound gave a violet-blue color with the spray reagent similar to the authentic amine.

The second component selected in the gas chromatographic screening had a retention time of 27.1 min. The infrared spectrum of the eluted peak was suggestive of an aryl amine and was subsequently found to be identical with 9,9-dimethylacridan (DMA). The mass spectrum of this component had a parent peak of 209

[1] *Whether or not an acridan is an aryl amine may be controversial. In the present report, it is so considered.*

and an intense fragment at 194 (loss of $-CH_3$) and was identical with that of DMA. Gas cochromatography on SE-30 of the unknown and authentic DMA gave a single peak.

The third component obtained in the screening had an elution time of 26.0 min. on SE-30. The mass spectrum of this unknown showed a parent peak at 211, an intense fragment at 196 (loss of $-CH_3$) and was similar to that of *N*-phenyl-4-isopropylphenylamine (PIA). The infrared spectra of PIA and the unknown were identical, and a single peak was obtained on cochromatography on SE-30.

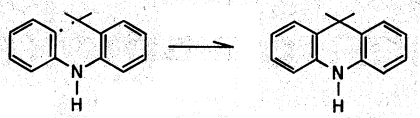
A major component was isolated in the gas chromatographic screening of fractions eluated from Column A with benzene, ether-benzene (1:4) and ether. The unknown had an infrared spectrum identical with N-phenyl-2-naphthylamine (PNA) and had m.p. 105°-107°C (lit., PNA: m.p. 108°C (11)). The mass spectrum showed an intense parent peak of 219 and was identical with that of authentic PNA. The ultraviolet spectra of the known and unknown amines were similar. Cochromatography of the isolated compound and PNA revealed a single peak on an SE-30 column with a retention time of 34 min. and a single spot on thin layer plates[2].

The isolated amounts (micrograms/cigarette) of DPA, DMA, PIA and PNA were 0.013, 0.04, 0.02 and 0.1, respectively.

A fifth compound was also isolated from fractions eluted from Column A. The isolate had infrared and mass spectra suggestive of a diisopropyldiphenylamine, but the amounts isolated were too small for conclusive identification.

The isolation of these amines from the neutral rather than the basic fraction is to be expected since they are feebly basic and would not be extracted into 6*N* HCl in the isolation procedure. Also, the isolation of these amines complements the previous identifications of aromatic compounds cited above and fortifies the concept that extraction of neutrals by nitromethane affords a method of concentrating many aromatic compounds in smoke condensate.

Except for aniline (3, 4), aryl amines have not been previously found in cigarette smoke or tobacco although the closely related β-phenylethylamine and methyl β-phenethylamine have been reported therein (3, 4). Regarding their origin in smoke, it is probable that they are formed in the burning process. If such is the case, other aryl amines may be present in smoke, such as the *ortho* isomer of *N*-phenyl-4-isopropylphenylamine. Formation of the isolated acridan from this isomer can be visualized easily by a free radical mechanism involving ring closure (I). Subsequent dehydrogenation and other reactions could lead to the formation of acridines known to (see I) be in cigarette smoke (10). This



I

route would serve as an alternate to the pyrolytic synthesis from pyridine alkaloids suggested by the studies of Van Duuren *et al.* (10).

In regard to biological activity, PNA is not tumorigenic (2). A report of papilloma formation by DPA (2) may not be reliable due to the presence of 4-aminobiphenyl in the tested material (1). Apparently, PIA and DMA have not been tested for biological activity.

Summary

Diphenylamine, 9,9-dimethylacridan, *N*-phenyl-4-isopropylphenylamine and *N*-phenyl-2-naphthylamine were identified in the nitromethane-soluble neutral fraction of cigarette smoke condensate. Identifications were made by spectral (infrared, ultraviolet and mass), chromatographic (gas and thin layer), and classical procedures.

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[2] *During the course of this study, other workers in this laboratory independently and concurrently isolated PNA from cigar smoke condensate (5).*